The aqueous root extract of *Aristolochia ringens* (Vahl.) Aristolochiaceae inhibits chemically-induced inflammation in rodents

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Abstract: The potential of the aqueous root extract of *Aristolochia ringens* (AR) (10-100 mg/kg p.o) to inhibit inflammation induced by phlogistics was evaluated using the carrageenan and egg albumin induced rat paw oedema, formaldehyde induced arthritic inflammation and xylene induced mouse ear oedema models. AR (10-50 mg/kg) dose-dependently decreased rat paw oedema in the carrageenan and egg albumin induced inflammation, producing comparable inhibition of 57.1% and 65.6% to the 57.9% and 63.9% of indomethacin and diclofenac (10 mg/kg p.o) respectively at 50 mg/kg. AR (10-50 mg/kg) also dose dependently inhibited formaldehyde-induced arthritic paw oedema over the 10 day observation period, with a greater inhibition of 50% at 50 mg/kg than the 40.8% inhibition by diclofenac (10 mg/kg i.p). AR (50 mg/kg) also produced greater inhibition of 84.78% than the 65.21% by dexamethasone (1 mg/kg) in xylene-induced ear oedema. Results show that the aqueous root extract of *Aristolochia ringens* possesses antiinflammatory activity.

Keywords: *Aristolochia ringens*, anti-inflammatory activity, carrageenan, prostaglandin, rodents.

INTRODUCTION

Inflammation is a protective defense mechanism of the body to disturbed homeostasis due to conditions such as infection and injury that result in systemic and local effects, signs of which may include pain, redness, swelling, heat and loss of function in the affected region. The inflammatory process is usually necessary for the removal of noxious stimuli and healing of wounds that may arise in the process, if uncontrolled, however, it may lead to the development of diseases. The combination of the complex mechanisms and mediators involved can induce and worsen inflammatory disorders such as rheumatoid arthritis and arteriosclerosis (Henson and Murphey, 1989), hence the need for anti-inflammatory drugs. So far the usefulness of available anti-inflammatory agents such as non-steroidal anti-inflammatory drugs (NSAIDs) and glucocorticoids has been limited by toxicity and resistance (Tapiero *et al*., 2002; Barnes and Adcock, 2009). In spite of the efforts made to combat some of these challenges, for instance, patients with glucocorticoid resistance can be treated with alternative broad-spectrum anti-inflammatory treatments, such as calcineurin inhibitors and other immunomodulators, or novel anti-inflammatory treatments, such as inhibitors of phosphodiesterase 4 or nuclear factor κB, there is still the challenge of these drugs being likely to have major side-effects (Barnes and Adcock, 2009). This, therefore, necessitates continual efforts to bring about effective management of inflammatory conditions. The investigation of medicinal plants reported to be efficacious in traditional medicine in the management of inflammatory disorders can be one of such efforts.

Aristolochia ringens (Vahl.) Aristolochiaceae is a bushy climber native to tropical America and introduced to most West African countries such as Sierra Leone and Nigeria, where it is used in traditional medicine for the management of rheumatoid arthritis, diarrhoea, snake bites and asthma (Olabanji *et al*., 2008; Sonibare and Gbile, 2008). Its anti-diarrhoeal activity has been demonstrated in our laboratory (Adeyemi *et al*., 2012). In spite of the reported potential toxicity of plants of the Aristolochiaceae family due to the presence of the slightly water soluble nitrophenanthrene carboxylic acid, aristolochic acid, *A. ringens* root extract is very commonly used medicinally in Nigeria though in small doses and for short periods. An evaluation of the safety of the plant is being undertaken in our laboratory. So far, the oral acute toxicity of its aqueous extract has shown that it is relatively safe on acute oral exposure as it did not produce any mortality or visible morbidity in the mice treated with up to 10,000 mg/kg according to Adeyemi *et al*. (2012), who also reported that the extract contains phyto-actives such as alkaloids, tannins, oils, saponin and reducing sugars, some of which have been reported to possess anti-inflammatory activities (Hosseinzadeh and Younesi, 2002).

The fact that other medicinal phytochemicals apart from aristolochic acid have been isolated from *Aristolochia* species (Pacheco *et al*., 2009), and β-naphthoflavone, a

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putative chemopreventive agent (Izotti *et al*., 2005) has been reported to prevent aristolochic acid-induced toxicity by enhancing its detoxification (Xiao *et al*., 2009) indicates that continuing research on promising species of these plants may allow for the optimization of their medicinal potentials. This study aimed to investigate the anti-inflammatory activity of the aqueous root extract of *A. ringens* (AR), to determine the pharmacological bases for its use in traditional medicine for the management of inflammatory conditions.

MATERIALS AND METHODS

Plant material

The root of *Aristolochia ringens* was collected from a local market in Mushin, Lagos, Nigeria and identified and authenticated by Mr T.K. Odewo, of the Department of Botany and Microbiology, University of Lagos, Nigeria where a herbarium specimen was deposited with voucher number LUH 4061.

Experimental animals

Adult albino mice (15-25 g) and rats (120-200 g) of either sex obtained from the Laboratory Animal Centre of the College of Medicine, University of Lagos, Nigeria were used for the experiment. The animals were kept under standard environmental conditions, allowed to feed and drink water *ad libitum*. Experimental procedures were carried out in accordance with the United States National Institute of Health Guidelines for Care and Use of Laboratory Animals in Biomedical Research (1985).

Extract preparation

Hundred grammes of the air dried root was soaked in 1 l of distilled water and placed in a refrigerator at 4^{o}C for 5 days. The liquid was decanted into a beaker of known weight and oven dried at 40^{o}C. Required concentrations were made from the extract just before the experimental procedures described below. The percentage yield of the extract was 4.2%.

Carrageenan-induced rat paw oedema

Carrageenan (0.1 ml of 1% w/v) dissolved in distilled water was injected into the sub-plantar region of the right hind paw of rats divided into 6 groups of 5 rats each. Paw size was measured before injection of carrageenan (C_0) and at intervals of 1, 2, 3, 4, 5 and 6 hours after carrageenan injection using the cotton thread method. AR (10, 25, 50 and 100 mg/kg), vehicle (10 ml/kg) and indomethacin (10 mg/kg) were orally administered to various groups respectively one hour before carrageenan injection. The mean increase in paw swelling was determined (Winter *et al*., 1962; Okpo *et al*., 2001; Bamgbose and Naomesi, 1981).

Egg albumin-induced rat paw oedema

Rats (6 groups of 5 rats each) of either sex were administered AR (10-100 mg/kg), indomethacin (10 mg/kg) or distilled water (10 ml/kg) 30 minutes before 0.1 ml of fresh undiluted egg albumin was injected into the sub-planter surface of the right hind paw of the rats. Paw sizes were measured before and 1, 2 and 4 hours after egg albumin injection (Akindele and Adeyemi, 2007; Anosike and Obidua, 2010).

Formaldehyde-induced inflammation

Rats (6 groups of 5 rats each) received 0.1 ml of 10% formaldehyde solution in normal saline in the plantar aponeurosis of the left hind paw on the first and third day of the study. The extract (10-100 mg/kg p.o), distilled water (10ml/kg p.o) or diclofenac (10 mg/kg i.p) were administered daily for 10 days. The rat paw circumference was measured daily for 10 days. The percentage inhibition of the mean increase in the paw oedema of each group was determined by the tenth day (Akindele and Adeyemi, 2007).

Xylene–induced ear oedema

Albino mice (6 groups of 5 mice each) were administered AR (10-100 mg/kg), dexamethasone (1 mg/kg) or distilled water (10ml/kg) orally. Thirty minutes later, inflammation was induced by topical application of 1 drop of xylene on the inner surface of the right ear. The animals were sacrificed 15 minutes later under ether anaesthesia and the left and right ears excised and made of equal sizes. The difference between the ear weights was taken as the oedema induced by the xylene (Adeyemi *et al*., 2002).

Effect of AR on castor oil induced-diarrhoea

Three groups of 6 mice each were treated with AR (50 mg/kg, p.o.), indomethacin (10 mg/kg, p.o.) or distilled water (10 ml/kg, p.o.) 30 minutes before administration of castor oil (0.2 ml/mouse). Each mouse was kept for observation under a glass funnel, the floor of which was lined with a white paper and observed for 4 hours. The parameter observed was the onset of diarrhoea (Mayureen and Ilavarasan, 2009). The diarrhoea score was also determined.

Statistical analysis

The results were reported as mean$\pm$standard error of mean (S.E.M) while statistical significance between groups was tested using the analysis of variance (ANOVA) followed by Tukey's multiple comparison test. Values were considered statistically significant at $p<0.05$.

RESULTS

Carrageenan-induced inflammation

AR (10-100 mg/kg) significantly reduced increase in rat paw size with a peak effect of 57.10% observed by the sixth hour at 25 mg/kg. This is comparable to the 57.90% reduction by indomethacin (10 mg/kg) at the fourth hour, by which time AR (50 mg/kg) produced a comparable 52.60% reduction (table 1).

Table 1: The effect of AR on carrageenan-induced rat paw oedema

Treatment Groups	Dose (mg/kg)	Increase in paw sizes (cm)					
		Hour 1	Hour 2	Hour 3	Hour 4	Hour 5	Hour 6
Vehicle (ml/kg)	10	0.40±0.04	0.51±0.06	0.56±0.06	0.57±0.06	0.46±0.06	0.35±0.06
AR	10	0.30±0.04 (25.00)	0.43±0.06 (15.70)	0.41±0.04 (26.80)	0.38±005 (33.30)	0.24±0.02[b] (47.80)	0.17±0.02[a] (51.40)
AR	25	0.25±0.02 (37.50)	0.39±0.04 (23.50)	0.37±0.02 (33.90)	0.28±0.03[b] (50.90)	0.23±0,02[b] (50.00)	0.15±0.02[a] (57.10)
AR	50	0.25±0.02 (37.50)	0.26±0.03 (49.00)	0.29±0.06 (48.20)	0.27±0.02[b] (52.60)	0.23±0.03[b] (50.00)	0.15±0.03[a] (57.10)
AR	100	0.38±0.05 (05.00)	0.47±0.07 (08.00)	0.37±0.05 (33.90)	0.31±0.04[b] (45.60)	0.33±0.04 (28.30)	0.29±0.03 (17.14)
Indomethacin	10	0.35±0.05 (12.50)	0.33±0.08 (54.50)	0.34±0.13 (39.30)	0.24±0.06[c] (57.90)	0.28±0.05[a] (39.10)	0.22±0.0 (37.10)

Values are mean ±SEM. [a]p<0.05, [b]p<0.01, [c]p<0.001 vs control. Values in parenthesis are percentage inhibition n=5

Table 2: The effect of AR on egg albumin-induced rat paw oedema

Treatment Group	Dose (mg/kg)	Increase in paw size (cm)		
		Hour 1	Hour 2	Hour 4
Vehicle (ml/kg)	10	0.88±0.10	0.77±0.08	0.61±0.08
AR	10	0.79±0.08 (10.20)	0.57±0.01[a] (26.00)	0.41±0.06 (32.70)
AR	25	0.73±0.05 (17.05)	0.51±0.04[b] (33.80)	0.33±0.06[a] (45.90)
AR	50	0.54±0.02[b] (38.60)	0.45±0.02[c] (41.50)	0.21±0.02[c] (65.60)
AR	100	0.62±0.05 (29.50)	0.50±0.03[b] (35.10)	0.21±0.02[c] (65.60)
Diclofenac	10	0.63±002 (28.40)	0.53±0.05[a] (31.20)	0.22±0.03[c] (63.90)

Values are mean ±SEM. [a]p<0.05,. [b]p<0.01, [c]p<0.001 vs control. Values in parenthesis are percentage inhibition n=5

Table 3: The effect of AR on formalin-induced rat paw oedema

Treatment group	Dose (mg/kg)	Increase in paw size (cm)	% Inhibition
Vehicle (ml/kg)	10	1.20±0.03	-
AR	10	0.94±0.03[c]	21.70
AR	25	0.84±0.03[c]	30.00
AR	50	0.60±0.03[c]	50.00
AR	100	0.78±0.03[c]	35.00
Diclofenac	10	0.71±0.03[c]	40.80

Values are mean ±SEM. [c]p<0.001 vs control. n=5

Table 4: The effect of AR on xylene-induced ear oedema

Treatment group	Dose (mg/kg)	Increased ear weight (mg)	% inhibition
Vehicle (ml/kg)	10	0.046±0.012	-
AR	10	0.026±0.004	43.40
AR	25	0.018±0.004[a]	60.86
AR	50	0.007±0.002[c]	84.78
AR	100	0.013±0.006[b]	71.74
Dexamethasone	1	0.016±0.002[a]	65.21

Values are mean ±SEM. [a]p<0.05, [b]p<0.01, [c]p<0.001 vs control. n=5

Table 5: The effect of AR in the test for delay in castor oil-induced diarrhoea

Treatment groups	Dose (mg/kg)	Diarrhoea onset (seconds)	Diarrhoea score
Vehicle (ml/kg)	10	50.17±3.49	30.67±3.83
AR	50	94.50±4.97[c]	20.17±2.17[a]
Indomethacin	10	235.30±4.67[c]	01.83±0.98[c]

Values are mean ±SEM, [a]p<0.05, [c]p<0.001 vs control n=6

Egg albumin-induced rat paw oedema
The extract (10-50 mg/kg) significantly reduced the paw size increase with a greater peak inhibition of 65.60% by the fourth hour at 50 mg/kg than the 63.90% reduction in paw size by diclofenac (10 mg/kg) (table 2).

Formalin-induced rat paw oedema
The extract (10-100 mg/kg), like diclofenac significantly ($p<0.001$) reduced paw size increase. The peak effect by the extract (50.00% inhibition) was greater than the 40.80% reduction by diclofenac (10 mg/kg) (table 3).

Xylene-induced mice ear oedema
AR (25-50 mg/kg) produced a significant reduction of ear size increase in the mice used. The peak effect of 84.78% reduction by the extract (50 mg/kg) was greater than the 65.21% reduction by dexamethasone (1 mg/kg) (table 4).

Effect of AR on Castor oil-induced diarrhoea
The extract (50 mg/kg), like diclofenac (10 mg/kg) produced a significant ($p<0.001$) delay in the onset of diarrhoea induced by castor oil. AR and diclofenac also significantly reduced the diarrhoea score (table 5).

DISCUSSION

A significant inhibition of carrageenan-induced rat paw oedema by AR was observed in the fourth to sixth hour period showing that AR may be more effective against mediators of the middle to late phases of the carrageenan-induced inflammation. Carrageenan, as a phlogistic is used classically in the investigation of NSAIDs such as indomethacin and diclofenac. According to Morris, (2003), inflammation via carrageenan consists of an early phase of histamine, serotonin and bradykinin release and a late phase of prostaglandin release. This indicates that AR, like NSAIDs, may possess inhibitory action on cyclooxygenase, the enzyme responsible for the synthesis of prostaglandins, which are potent mediators of inflammation. In addition, the second phase of carrageenan-induced inflammation has also been reported to involve neutrophil infiltration and production of reactive free radical species derived from them (Dordevic *et al*., 2007; Sofidiya *et al*., 2010). This indicates that AR may also exert its anti-inflammatory activity through the inhibition of neutrophil infiltration, prevention of free radical generation, and/or enhancement of free radical scavenging. AR (10-50 mg/kg) also appears to possess a relatively longer duration of action than indomethacin as it produced significant and greater percentage inhibition up to the 6^{th} hour.

The egg albumin has been reported to produce mast cell degranulation with subsequent release of inflammatory mediators such as histamine, which has been associated with increased vasodilatation and increased permeability of blood vessels leading to the exudation of plasma proteins and fluids into the tissues (Anosike and Obidun, 2010; Harriot *et al*., 2004), hence the oedema. In this model, AR (10-50 mg/kg) also produced a dose dependent reduction of the rat paw size increase induced by egg albumin. The peak effect of the extract (50 mg/kg) observed in the fourth hour was higher than that of diclofenac (10 mg/kg). This effect of the extract may be due to mechanisms such as mast cell stabilization and/or anti-histaminergic activity.

The formaldehyde test is sometimes used as a model for the investigation of arthritis related tissue damage (Brownlee, 1950;Singh *et al*., 2011) leading to the release of inflammatory mediators including neuropeptides such as susbstance P and mast cell amines such as histamine and serotonin (Damas and Liegeolis, 1999). Its result in this study has shown that the extract is capable of alleviating arthritic and subchronic inflammation.

The xylene-induced ear oedema test, which is more sensitive to steroidal anti-inflammatory drugs e.g. dexamathasone, which inhibits phospholipase A_2 (Zaninir *et al*., 1992; Hirata *et al*., 1980) has also shown that the extract possesses steroidal antiinflammatory activity. The test for the delay in castor oil-induced diarrhoea has been reported as useful for determining inhibition of prostaglandin by test compounds as a mechanism for anti-inflammatory action (Mayureen and Ilavarasan, 2009). In this study, the extract shows a possible inhibitory role in the synthesis and/or activity of prostaglandin.

Given the fact that aristolochic acid is only slightly soluble in water, the method of extraction and the efficacy of the *A. ringens* extract in this study; *A. ringens* appears to possess important components (other than aristolochic acid) responsible for its action. Indeed an investigation in our laboratory, using fractions of the extract in which aristolochic acid was undetectable, showed significant anti-inflammatory activity by the fractions (data not shown). However, there is need for caution, given the toxic reports of plants in this genus. The toxicological investigation and phytochemical analysis of this aqueous preparation are currently ongoing in our laboratory.

CONCLUSION

These results show that the aqueous root extract of *A. ringens* is active against phlogistics-induced inflammation and provides a basis for the reported efficacy of *A. ringens* in traditional herbal medicine for relief of inflammatory conditions.

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REFERENCES

Adeyemi OO, Okpo SO and Ogunti OO (2002). Analgesic and anti-inflammatory effects of *Persia americana* Mill (Lauraceae). *Fitoterapia*, **73**: 375-380.

Adeyemi OO, Aigbe FR and Badru OA (2012). The antidiarrhoeal activity of the aqueous root extract of *Aristolochia ringens* (Vahl.) Aristolochiaceae. *Nig. Qt. J. Hosp. Med.,* **22**(1): 29-33.

Akindele AJ and Adeyemi OO (2007). Anti-inflammatory activity of the aqueous leaf extract of *Byrsocarpus coccineus. Fitoterapia*, **78**: 25-28.

Anosike OA and Obidua O (2010). Anti-inflammatory and anti-ulcerogenic effect of ethanol extract of coconut (*Cocos nucifera*) on experimental rats. *AJFAND*., **10**(10): 4286-4300.

Bamgbose SOA and Naomesi BK (1981). Studies on cryptolepine II: Inhibition of carrageenan induced oedema by cryptolepine. *Planta medica.*, **42**: 392-396.

Barnes PJ and Adcock IM (2009). Glucocorticoid resistance in inflammatory disease. *Lancet*, **373**: 1905-1917.

Brownlee G (1950). Effect of deoxycortisone and ascorbic acid on formaldehyde-induced arthritis in normal and adrenalectomized rats. *Lancet*, **1**: 157-159.

Damas J and Liegeolis J (1999). The inflammatory reaction induced by formalin in the rat paw. *Naunyn-Schmiedeberg's Arch Pharmacol.*, **359**: 220-227

Dordevic S, Petrovi´c S, Dobri´c S, Milenkovi´c M, Vu˘ci´cevi´c D, Zi˘zi´c S and Kuki´ J (2007). Antimicrobial, anti-inflammatory, anti-ulcer and antioxidant activities of *Carlina acanthifolia* root essential oil. *J. Ethnopharmacol*., **109**: 458-463

Harriot M, Marion E, Martha A, Wellford S and William A (2004). Inflammation induced by histamine, serotonine, bradykinin and compound 48/480 in the rat, antagonists and mechanisms of action. *J. Pharmacol. Exp. Ther*., **191**: 300-302.

Henson PM and Murphey RC (1989). Mediators of the inflammatory process. Elsevier, Amsterdam, p.404.

Hirata F, Schuffman E and Venkatasubramamian K (1980). A phospholipase A_2 inhibitory protein in rabbit neutrophils induced by glucocorticoids. *Proc. Natl. Acad. Sci.,* **77**: 2533-2536.

Hosseinzadeh H and Younesi HM (2002). Antinociceptive and anti-inflammatory effects of *Crocus sativus* L. stigma and petal extracts in mice. *BMC Pharmacology*, **2**: 7-14.

Izotti A, Bagnasco M, Cartiglia C, Longobardi M, Camoirano A, Tampa E, Lubet RA and De Flora S (2005). Modulation of multigene expression and proteome profiles by chemopreventive agents. *Mutat. Res.*, **591**(1-2): 212-223.

Mayureen C and Ilavarasan R (2009). Anti-inflammatory activity of ethanolic leaf extract from *Adenanthera pavonina* (L.) in rats. *J. Young Pharm*., **1**: 125-128.

Morris CJ (2003). Carrageenan-induced paw oedema in the rat and mouse. *Methods Mol. Biol.,* **225**: 115-121.

Okpo SO, Fatokun F and Adeyemi OO (2001). Analgesic and anti-inflammatory of *Crinum glaucum* aqueous extract. *J. Ethnopharmacol.,* **78**: 207-211.

Olabanji SO, Omobuwajo OR, Ceccato D, Adebajo AC, Buoso MC and Moschini G (2008). Accelerator-based analytical technique in the study of some anti-diabetic medicinal plants of Nigeria. *Nucl. Instr and Meth. in Phys. Res. B*., **266**: 2387-2390.

Pacheco AG, Machado de Oliveira P, Piló-Veloso D and Alcântara AF (2009). 13C-NMR Data of diterpenes isolated from *Aristolochia* species. *Molecules.*, **14**: 1245-1262.

Singh S, Nair V and Gupta YK (2011). Antiarthritic activity of "Majoon Suranjan" (a poly herbal Unaniformulation) in rat," *Indian J. Med. Res*., **134**: 384-388.

Sofidiya MO, Odukoya OA, Adedapo AA, Mbagwu HOC, Afolayan AJ and Familoni OB (2010). Investigation of the anti-inflammatory and anti-nociceptive activities of *Hymenocardia acida* Tul. (*Hymenocardiaceae*). *Afr. J. Biotechnol*., **9**(49): 8454-8459.

Sonibare MA and Gbile ZO (2008). Ethnobotanical survey of anti-asthmatic plants in Southwestern Nigeria. *AJTCAM.*, **5**(4): 340-345.

Tapiero H, Ba NG, Couvreur P and Tew KD (2002). Polyunsaturated fatty acids (PUFA) and eicosanoids in human health and pathologies. *Biomed. Pharmacother.*, **56**: 215-222.

United States National Institute for Health Publications (1985). No. 85, p.23.

Winter CA, RiselyEA and Nuss GW (1962). Carrageenan induced oedema in hind paw of the rat as an assay for anti-inflammatory drugs. *Proc. Soc. Exp. Biol. Med.,* **11**: 533-547.

Xiao Y, Xue X, Wu YF, Xin GZ, Qian Y, Xie TP, Gong LK and Ren J (2009). Beta-naphthoflavone protects mice from aristolochic acid-I-induced acute kidney injury in a CYP1A dependent mechanism. *Acta. Pharmacol. Sin.*, **11**: 1559-1565.

Zaninir JC, Medeiro YS, Cruz AB, Yones RRA and Calixto JB (1992). Action of compounds from *Mandevilla velutina* on croton oil induced ear oedema in mice: A comparative study with steroidal and non-steroidal anti-inflammatory drugs. *Phytother. Res.,* **6**: 1-5.